DESCRIPTIONS OF THREE SPECIES OF *EUPLOTES* (PROTOZOA: CILIATEA)

By C. R. CURDS

INTRODUCTION

In his revision of the order *Hypotrichida* Stein, 1859, Borror (1972) listed 43 species of the genus *Euplotes* Ehrenberg, 1830 and in the same year Carter (1972) added a further four new species. The latter author suggested the following characters constitute a reliable basis for separating species within the genus ; the pattern of the dorsal interkinetal silver-line network or argyrome, the number of dorsolateral kineties, the shape of the adoral zone of membranelles and the number of membranelles therein, the number and arrangement of the ventral cirri and the shape of the non-dividing macronucleus. Most of these characters were initially introduced by Tuffrau (1960) while Borror (1968) added the appearance of the cortical sculpturing that is sometimes a feature of the dorsal and ventral surfaces of *Euplotes* spp.

Three species of *Euplotes* are described in the present paper. The first two are freshwater forms that were isolated from samples of activated sludge and these were subsequently identified as *E. moebiusi* forma *quadricirratus* and *E. affinis* forma *tricirratus* respectively. The third is a euryhaline species from Austria which does not conform to any of those species described in the literature when both traditional and modern criteria are taken into consideration.

Euplotes moebiusi Kahl, 1932 and the variety with four caudal cirri, *E. moebiusi* forma *quadricirratus* Kahl, 1932, have not been described in the literature since their first brief descriptions and illustrations by Kahl (1932). However, this species has regularly been observed in activated-sludge plants treating sewage and industrial waste waters over many years (Curds & Cockburn, 1970 ; Ministry of Technology 1968). Photographs of *E. moebiusi* were published by Klein (1958) in order to demonstrate the 'dry' silver method but these were not sufficiently comprehensive for taxonomic purposes.

Euplotes affinis Dujardin, 1841 and its variety with three caudal cirri *E. affinis* forma *tricirratus* Kahl, 1932 are also examples that have not been described since their originals and yet have been seen regularly in aerobic waste-treatment processes (Curds & Cockburn, 1970) and in other organically polluted situations (Bick, 1972). Tuffrau (1960) thought it likely that both *E. moebuisi* and *E. affinis* were synonyms for the species *E. charon* Ehrenberg, 1830.

MATERIALS AND METHODS

(a) *Source and cultivation*

A clonal culture of *Euplotes moebiusi* was isolated direct from an activated-sludge sample obtained from Maple Lodge Sewage Treatment Works, Rickmansworth, Hertfordshire. This species was maintained in freshwater Erdschreiber solution

Bull. Br. Mus. nat. Hist. (Zool.) **27**, 2

('Medium 1', Committee on Cultures, Society of Protozoologists, 1958), either in test-tubes or in Petri dishes. The largest populations were obtained in Petri dishes containing a thin layer of Musgrave and Clegg's agar (2·5 per cent agar, 0·5 per cent sodium chloride and 0·5 per cent Liebig's beef extract in distilled water) which was streaked with the bacterium *Klebsiella aerogenes* (National Collection of Industrial Bacteria, NCIB 8017) as a food supply and then flooded with Erdschreiber solution. Subcultures were transferred at monthly intervals.

Euplotes affinis was collected by Mr A. Cockburn from a sample of activated sludge taken from an experimental small-scale pilot plant operated at the Water Pollution Research Laboratory, Stevenage, Hertfordshire. It was sent to the British Museum (Natural History) as a clonal culture and was maintained in a similar manner to *E. moebiusi*.

The third hypotrich, a small euryhaline *Euplotes* sp., was originally collected by Professor E. Tschermak from a freshwater source in Schlospark Schönbrunner in Vienna which is the *locus classicus* for the alga *Ruttnera spectabilis* Geitler, 1942 (see Geitler, 1942, 1943). Samples of this alga were sent to Dr Mary Parke at the Marine Biological Station at Plymouth where the hypotrich was first noticed and cultured. Cultures of the ciliate were subsequently deposited with the NERC Culture Collection of Algae and Protozoa where it was cultivated in saltwater Erdechreiber solution. Later, a clonal culture was established in freshwater and marine media at the British Museum (Natural History) and the descriptions herein relate to organisms from that clone. This small *Euplotes* sp. could be maintained equally well in test-tubes or plastic Petri dishes containing either fresh or seawater Erdschreiber solutions. Cultures were kept in the dark at room temperature, and were fed at weekly intervals with a few drops of a thick suspension of baker's yeast. Cultures were transferred at monthly intervals.

(b) *Microscopy*

Light microscopy and the methods used for observations and measurements were similar to those described by Curds, West & Dorahy (1974). Silver-line systems were displayed using the 'wet' method of Corliss (1953) in the cases of *Euplotes affinis* and the small euryhaline species. The 'dry' method of Klein (1958) was used to show the silver-line system of *E. moebiusi*. The latter method proved to be far more reliable and quicker than the conventional 'wet' method. As Klein (1958) pointed out, the success of the 'dry' method depends on the cell drying and dying more or less simultaneously and this was achieved by removing excess moisture with the aid of screws of paper tissue and by flicking single cells out from drops of liquid onto dry parts of the slide by means of an eyelash mounted in a glass rod. Nuclei were stained using Dippell and Chao's modification of De Lamater's basic fuchsin method described by Sonneborn (1950). The nuclei of *E. moebiusi* and *E. affinis* were stained after fixation on the slide by air-drying with equal success as the conventional method of chemical fixation.

The techniques used for scanning-electron microscopy of the small euryhaline *Euplotes* sp. were similar to those previously described by Curds *et al.* (1974) with the exception of the fixation methods. Here the hypotrichs were not killed in osmium

tetroxide vapour, and fixation was best using the osmium-meruric chloride fixative (Parducz, 1967) which was recommended by Small & Maraszalek (1969). A comparison of the results obtained by the fixation methods of Curds *et al.* (1974) and those of Small & Marszalek (1969) for this species is demonstrated in Pl. 1. Plate 1a shows a cell fixed in Parducz's solution following the recommendations of Small & Marszalek (1969) where Pl. 1b shows a similar cell that had been killed in osmium vapour fixed in a solution containing equal parts of 2 per cent (w/v) osmium tetroxide and saturated mercuric chloride solutions. It is apparent that the Parducz's fixative was far better for this species than were the methods of Curds *et al.* (1974),whereas the reverse was true for the species *Euplotes rariseta* Curds, West & Dorahy, 1974. These results suggest therefore that the choice of fixative may vary with the species under consideration.

RESULTS

Euplotes moebiusi Kahl, 1932

Diagnosis. Medium (60 μm long, 40 μm wide), ovoid freshwater hypotrich with 10 frontoventral, 5 transverse and 4 caudal cirri. Ventral surface heavily sculptured with 7 ridges, dorsal surface with 5 longitudinal ridges. Adoral zone with 35–40 membranelles which extend two-thirds the length of the cell. Dorsal silver-line system with 5 longitudinal rows of narrow polygons interspersed with an irregular network of larger polygons ; 7 dorsolateral kineties bearing a maximum of 11 dorsal cilia. Macronucleus **3**-shaped, micronucleus small, situated anteriorly.

Slides showing silver-line systems, ventral ridges and nuclei have been deposited in the slide collection of the B.M. (N.H.), Reg. Nos. 1973:4:14:1–5.

Detailed description. It can be seen from the size distribution data given on Fig. 1 that *Euplotes moebiusi* is a medium-sized species and is $62{\cdot}25 \pm 6{\cdot}6$ μm long and $39{\cdot}45 \pm 5{\cdot}87$ μm wide. The outline shape of the body is oval and there is a definite notch at the anterior end of the body where the adoral zone of membranelles (AZM) begins. The ventral surface is heavily sculptured with 7 ridges. (Fig. 2). One flattened ridge runs along the edge of the peristome and terminates posteriorly in a sharp point. One short ridge is restricted to the anterior half of the body and lies between the front-ventral cirri separating cirrus streak I and II from streak III (using the method of cirrus numeration of Wallengren, 1900). Three short ridges are confined to the posterior and lie between the transverse cirri. One ridge stretches the entire length of the body beginning at the anterior notch (between streaks III and IV) and terminating between the transverse cirri III 1 and IV 1. One medium length ridge is restricted to the central portion of the body and separates cirri V 3 and VI 2 from cirrus V 2. The positions and shapes of these ridges are similar to those figured by Kahl (1932). There are 5 longitudinal ridges on the dorsal surface.

Euplotes moebiusi has 10 frontoventral cirri which are distributed as shown in Figs. 2 and 3b. The arrangement resembles that of *E. charon*. There are 5 transverse and 4 caudal cirri. No specimens were observed with 3 caudal cirri as was shown in the original descriptions by Kahl (1932). The AZM is composed of 35–40 membranelles and its extends two-thirds of the way down the body (Fig. 2).

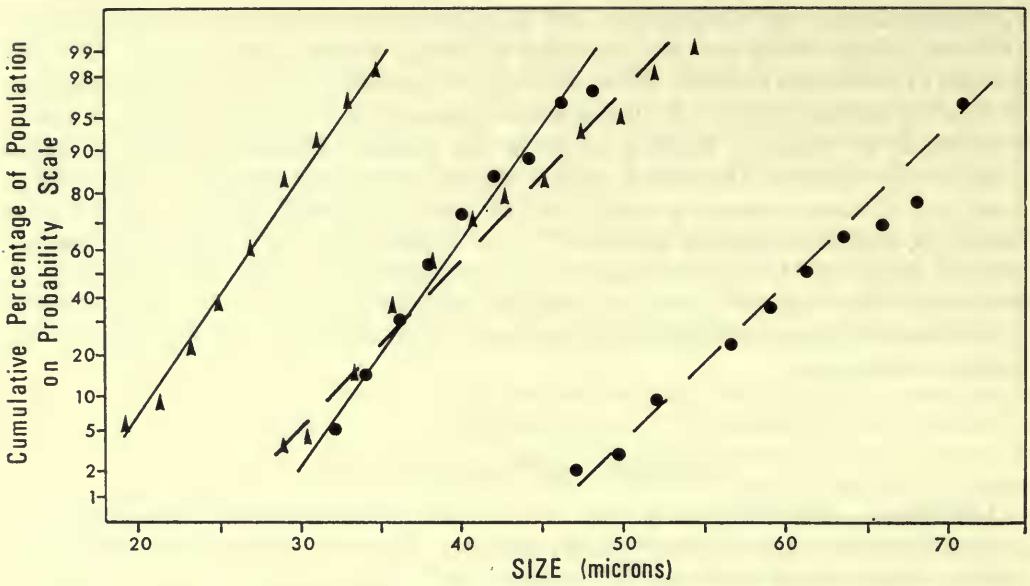

FIG. 1. Size distribution data of *Euplotes moebiusi* (broken lines) and *Euplotes affinis* (continuous lines). Triangles denote width of cells, circles denote length.

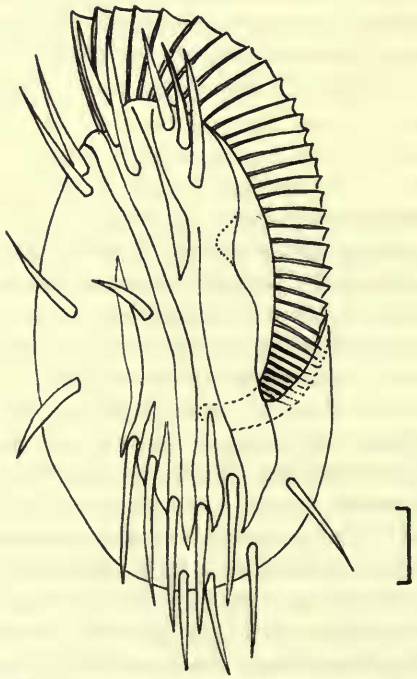

FIG. 2. Ventral aspect of *Euplotes moebiusi* showing cirri and ridges (scale represents 10 μm).

The geometry of the dorsal silver-line system of *Euplotes moebiusi* differs from any of those published. It consists (Fig. 3a) of 5 ladder-like longitudinal rows of narrow polygons with the pits of the dorsal cilia or bristles positioned on the right. In other *Euplotes* spp. with a double *patella*-type of dorsal argyrome the dorsal pits are situated on the left of the rows of smaller polygons. In addition, an irregular network of polygons, resembling that of *E. mutabilis* Tuffrau, 1960, is sandwiched between the ladder-like rows. There are 7 dorsolateral kineties with the central kineties bearing a maximum of 11 dorsal bristles. The ventral silver-line network (Fig. 3b) is a conventional series of irregular polygons whose general pattern closely resembles that of *E. patella* Muller, 1773 (see Tuffrau, 1960).

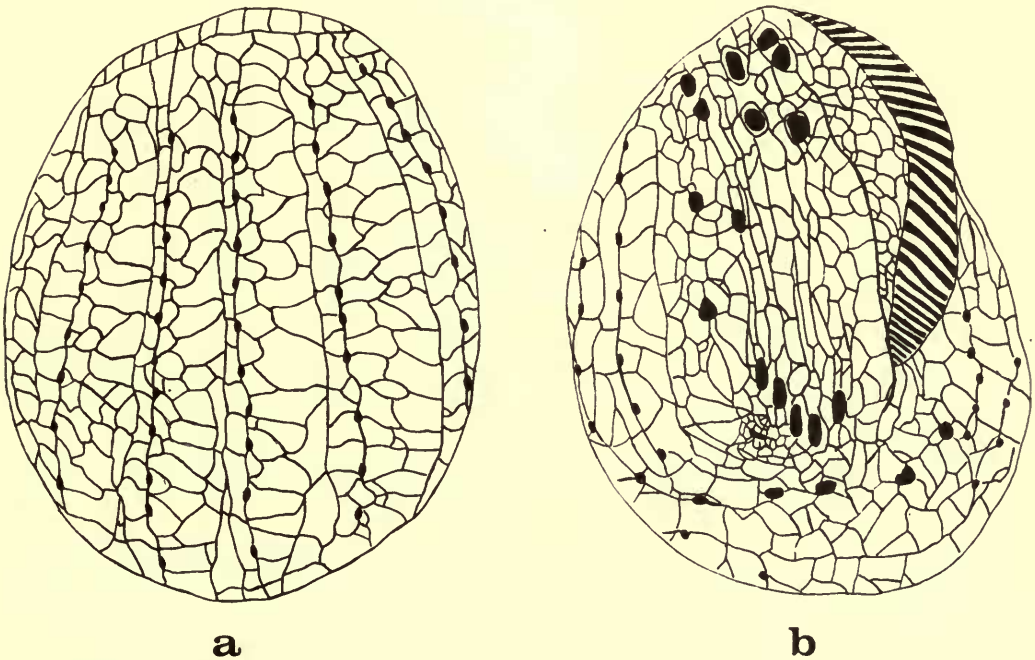

FIG. 3. Silver-line system of *Euplotes moebiusi*, (a) dorsal surface, (b) ventral surface.

The macronucleus of *Euplotes moebiusi* is an irregular **3**-shape (Fig. 4) resembling that of *E. plumipes* Stokes, 1884 (see Tuffrau, 1960), except that the posterior tail is shorter than in that species. The micronucleus is small and lies very close to, and sometimes overlaps, the left anterior edge of the macronucleus.

Euplotes affinis Dujardin, 1842

DIAGNOSIS. Small (38 μm long, 26 μm wide), ovoid freshwater hypotrich with 9 frontoventral, 5 transverse and 3 caudal cirri. Ventral surface sculptured with 3 prominent ridges and dorsal surface with 5 longitudinal ridges. AZM with 18–20

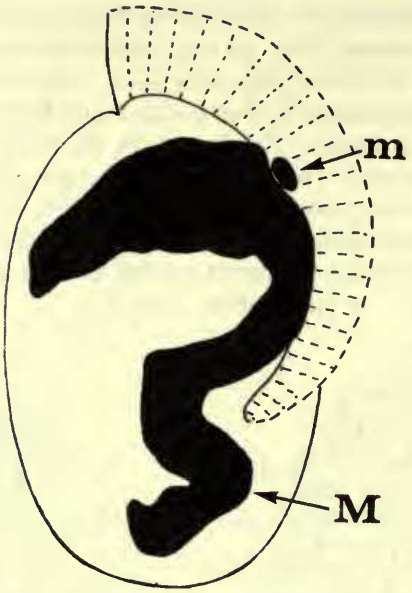

FIG. 4. Ventral view of the nuclei of *Euplotes moebiusi* from a stained preparation (M = macronucleus, m = micronucleus).

membranelles which extends two-thirds the length of the cell. There is a small undulating membrane. Dorsal silver-line system of the double *eurystomus* type with 7 dorsolateral kineties and a maximum of 9 dorsal cilia in the central kineties. The macronucleus is **3**-shaped and there is a small anterior micronucleus.

Slides showing silver-line systems and nuclei have been deposited in the slide collection of the B.M. (N.H.), Reg. Nos. 1973:9:26:1–10.

DETAILED DESCRIPTION. *Euplotes affinis* is one of the smaller freshwater species whose dimensions are $38{\cdot}4 \pm 4{\cdot}3$ μm long and $25{\cdot}8 \pm 4{\cdot}0$ μm wide. The size distribution data of this species are compared with those of *E. moebiusi* in Fig. 1. The outline shape of *E. affinis* resembles that of *E. moebiusi* and there is a marked notch at the anterior of the cell which denotes the origin of the AZM. The ventral surface is heavily sculptured by 3 longitudinal ridges (Fig. 5) which travel almost the entire length of the cell. The outer pair of ventral ridges flank the transverse cirri at the posterior end of the body and terminate anteriorly between cirrus streaks I and II and between streaks V and VI. There are 3 other minor ridges that are restricted to the posterior end of the cell and these lie between the transverse cirri. The positions and shapes of the complete ventral ridging conforms closely with those figured by Kahl (1932). The dorsal surface is also heavily sculptured with 5 longitudinal ridges.

Euplotes affinis has 9 frontoventral cirri whose distribution is shown in Figs. 5 and 6b. There are 5 transverse cirri and 3 caudal cirri ; one of the caudals is larger than the others and is held out stiffly to the right in a manner similar to that of *E. rariseta* (see Curds *et al.* 1974). No specimens have been observed with 4 caudal

cirri as was shown in the original descriptions of *E. affinis* by Dujardin (1841), although Kahl (1932) described the variety *E. affinis* forma *tricirratus* which had 3 caudal cirri. The AZM of *E. affinis* extends two-thirds the length of the body and is composed of 18–20 membranelles which is approximately half the number found in *E. moebiusi.*

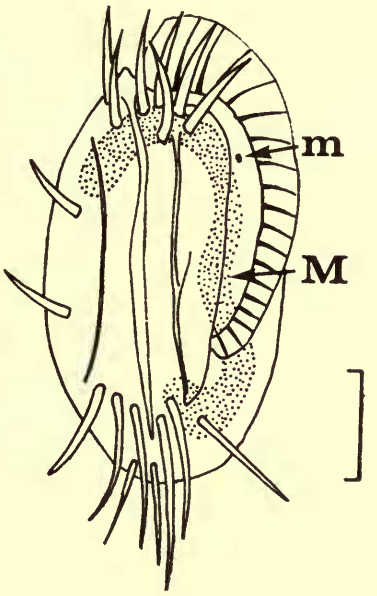

FIG. 5. Ventral aspect of *Euplotes affinis* showing cirri, ridges and nuclei (scale represents 10 μm, M = macronucleus, m = micronucleus).

The dorsal and ventral silver-line systems are shown in Fig. 6. The geometry of the dorsal argyrome (Fig. 6a) is of the double type resembling that of *Euplotes eurystomus* Wrzesniowski, 1870. There are 7 dorsolateral kineties in *E. affinis* and the central kineties bear a maximum of 9 dorsal cilia. The ventral silver-line system is of a common type consisting of a series of relatively few polygons.

The macronucleus is **3**-shaped and resembles those of *Euplotes harpa* Stein, 1859 and *E. plumipes* (see Tuffrau, 1960). The micronucleus is small and is situated at the anterior edge of the macronucleus.

Euplotes parkei sp. n.

DIAGNOSIS. Small (41 μm long, 30 μm wide) euryhaline species ; broadly oval in outline. Dorsal surface with 6 low longitudinal ridges and ventral surface with 7 minor ridges. AZM approximately two-thirds body length, composed of 18 membranelles. A deep pocket near the cytostome bears an undulating membrane. Usually 8, but rarely 9, frontoventral cirri ; 5 transverse and 4 caudal cirri. There are 8 dorsolateral kineties with a maximum of 11 dorsal cilia in the central kineties.

Dorsal silver-line system of the double *eurystomus*-type. Macronucleus C-shaped with anteriorly situated micronucleus.

Type slides showing silver-line systems and nuclei have been deposited in the slide collection of the B.M. (N.H.). Holotype Reg. No. 1973:4:2:1, and paratype Reg. Nos. 1973:4:2:2–5.

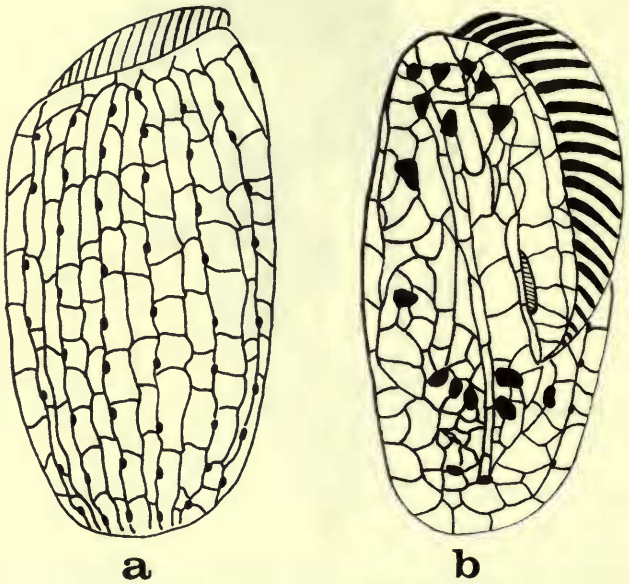

FIG. 6. Silver-line system of *Euplotes affinis*, (a) dorsal surface, (b) ventral surface.

DETAILED DESCRIPTION. This is a small euryhaline species (41·2 ± 5·6 μm long, 30·6 ± 5·3 μm wide) whose size distribution data are given in Fig. 7. It is broadly oval in outline shape with the dorsal surface sculptured with 6 relatively low longitudinal ridges. To the left of each ridge is a parallel row of pits from which the short (2 μm) dorsal bristles emerge (Pl. 1, figs. c & d). The ventral surface also bears 7 longitudinal ridges (Pl. 1, fig. a) but these are not as prominent as in *Euplotes moebiusi*. One ventral ridge travels the complete length of the body along the extreme edge of the peristome, while the other 6 are relatively short and are confined to the posterior half of the cell. The transverse cirri arise from between these 6 minor ridges. The AZM extends two-thirds the length of the body and is composed of 18 membranelles. There is an undulating membrane situated in a deep pocket on the right of the peristome in the proximity of the cytostome.

This species of *Euplotes* usually bears 8 frontoventral cirri which are arranged as shown in Figs. 8, 9b and Pl. 1, fig. a ; however, a 9th frontoventral cirrus is occasionally present within the same clone and this lies in a position V 2 (Pl. 1, fig. b). The 9th frontoventral cirrus has been found only in animals cultured in freshwater Erdschreiber solution even though the marine cultures have been searched thoroughly

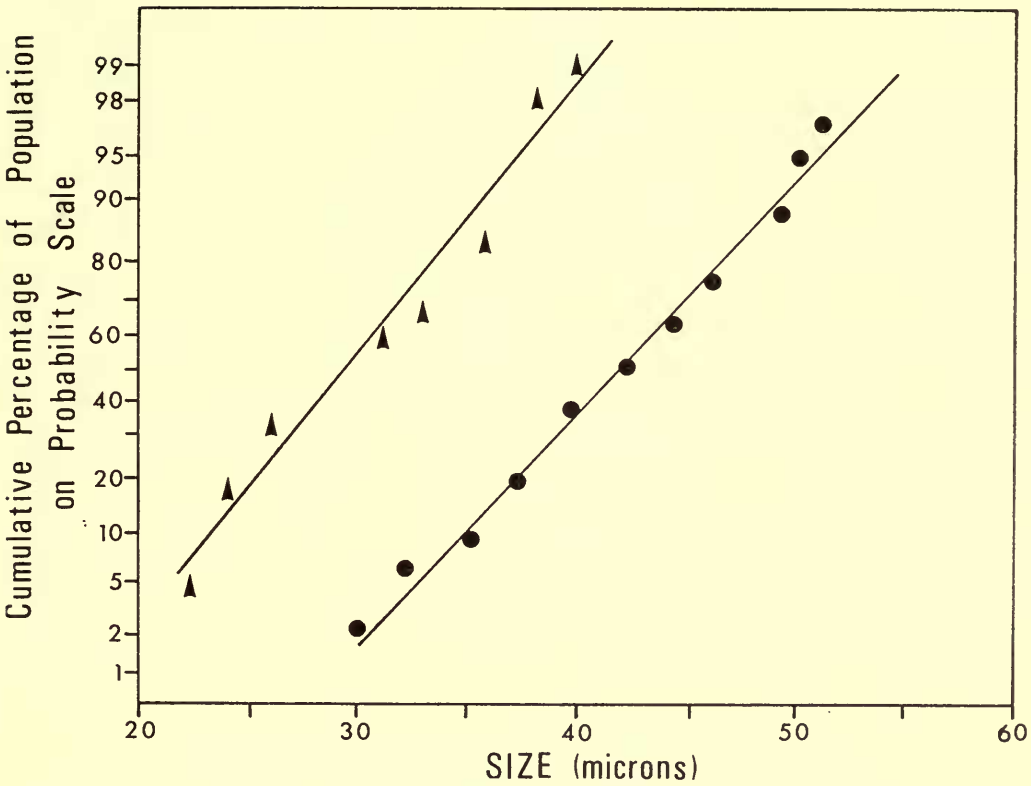

FIG. 7. Size distribution data of *Euplotes parkei*. Triangles denote width and circles length of the cells.

for this variant. It is not yet known if the salinity of the culture plays any part in promoting this type of intraspecific polymorphism. The 5 long transverse and 4 caudal cirri were constant in their numbers.

There are 8 dorsolateral kineties in this species and the central kineties bear a maximum of 11 dorsal cilia. The dorsal and ventral silver-line systems are shown in Fig. 9. The disposition of the dorsal argyrome (Fig. 9a) is of the double type resembling that of *Euplotes eurystomus*. The ventral silver-line system consists of a series of few but large polygons and in this respect resembles that of *E. cristatus* Kahl, 1932 (see Tuffrau, 1960). The dorsal argyrome can be seen on some scanning-electron micrographs (Pl. 1, figs. c & d) as a series of tiny specks. The macronucleus (Fig. 8) is C-shaped and the micronucleus is situated close to the left anterior edge of the macronucleus.

DISCUSSION AND CONCLUSIONS

Euplotes moebiusi is one of the nine species of the genus whose silver-line system had not been fully described, and its identity relied solely upon the brief description of Kahl (1932). It is evident from the description of the silver-line system given in

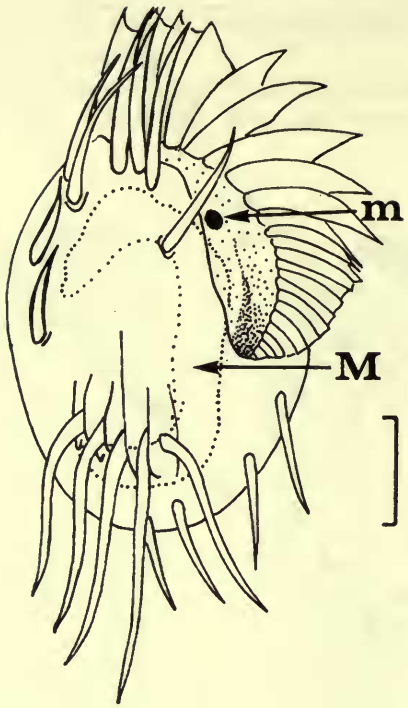

FIG. 8. Ventral aspect of *Euplotes parkei* showing cirri, ridges and nuclei (scale represents 10 μm, M = macronucleus, m = micronucleus).

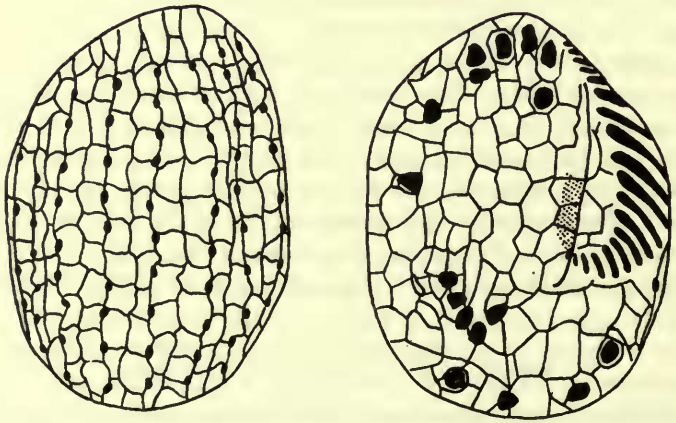

FIG. 9. Silver-line system of *Euplotes parkei*, (a) dorsal surface, (b) ventral surface.

the present paper that *E. moebiusi* is a distinct species and is not a synonym of *E. charon* as was suggested by Tuffrau (1960).

Before the completion of the present studies it was the opinion of the author that *Euplotes moebiusi* was likely to be a synonym of *E. affinis* because of the similarities between the two species which may be listed thus ; they both have the same general shape and size with a definite anterior notch ; both have ventral ridges of similar appearance ; both *E. moebiusi* and *E. affinis* are reported to have 4 and 3 caudal cirri variants called *E. moebiusi* forma *quadricirratus* and *E. affinis* forma *tricirratus* respectively ; both have **3**-shaped macronuclei and both may be found in organically polluted freshwater habitats. This meant that there was only one known character left to separate the two species, namely the presence or absence of frontoventral cirrus V 2. In view of the findings presented in this paper concerning the intraspecific polymorphism of *E. parkei* due to the variability of cirrus V 2 it is evident that the presence or absence of this cirrus is not as reliable a character as was originally believed. The results presented here however have clarified the situation considerably and *E. affinis* can now be distinguished from *E. moebiusi* by the 5 characters listed in Table 1. The most reliable character is the geometry of the dorsal silver-line system which is completely different in the two species (compare Figs. 3b & 6b). In the author's opinion *E. affinis* should not be regarded as a synonym of *E. charon* as was suggested by Tuffrau (1960), and there are sufficient reliable characters to regard *E. affinis* as a species distinct from all others.

TABLE 1

List of characters which serve to distinguish between *Euplotes moebiusi* and *Euplotes affinis*

Character	*E. moebiusi*	*E. affinis*
Number of frontoventral cirri	10	9
Number of membranelles in AZM	35–40	18–20
Maximum number of dorsal cilia in mid-dorsolateral kineties	11	9
Dorsal argyrome	Complex	Double *eurystomus*
Ventral argyrome	Many small polygons	Few large polygons

Agamaliev (1967) reported intraspecific polymorphism in the number of frontoventral cirri in his description of *Euplotes raikovi* Agamaliev, 1966. In the Caspian Sea strain of *E. raikovi*, Agamaliev (1967) noted that there were 7 or 8 frontoventral cirri and indicated that cirrus V 2 was that which did not develop in some specimens. However in a recent paper, Washburn & Borror (1972) described a strain of *E. raikovi*, isolated from a sand sample taken from the New Hampshire coast of the U.S.A., in which they could not find an 8th cirrus (cirrus V 2) although they did observe a completely barren plaque in each case. Negative evidence such as this can never be conclusive and one must accept that Agamaliev's strain did exhibit polymorphism of cirrus V 2 as he claimed, particularly in the light of the photographic evidence presented in this paper where there can be no doubt that *E. parkei* may have 8 or 9 completely normal frontoventral cirri. More work must be carried

out on the morphogenesis of *E. parkei* particularly on the fate of cirrus V 2, but the evidence so far obtained indicates that there is not even a barren plaque in the case of *E. parkei* specimens with 8 frontoventral cirri. Furthermore, more work is needed to test adequately whether or not intraspecific polymorphism such as this can be induced by adjusting the salinity of the culture medium or if the results so far obtained can be attributed to pure chance.

It is possible that one of the reasons why *Euplotes parkei* has remained unnoticed until now is because none of its characters, in isolation, will distinguish this species from all others. A combination of characters is required to do so, but there can be little doubt that this small euryhaline *Euplotes* is a separate and distinct species. The following species have the combination of characters – a double dorsal argyrome with 8 or 9 frontoventral, 5 transverse and 4 caudal cirri – *E. aediculatus* Pierson, 1943 ; *E. amieti* Dragesco, 1970 ; *E. aspheronicus* Agamaliev, 1966 ; *E. diadaleos* Diller & Kounaris, 1966 ; *E. eurystomus* ; *E. octocarinatus* Carter, 1972 ; *E. patella* ; *E. patella* forma *latus* Agamaliev, 1967 ; *E. plumipes* ; *E. tegulatus* Tuffrau, 1960 ; *E. tuffraui* Berger, 1965 ; *E. variabilis* Stokes, 1887 (see Carter, 1972) ; and *E. zenkewitchi* Burkovsky, 1970. However only four of these, *E. aediculatus*, *E. eurystomus*, *E. variabilis* and *E. octocarinatus*, have 8 dorsolateral kineties. Since *E. octocarinatus* has a *patella*-like double dorsal argyrome there are only three remaining species with which to compare and contrast *E. parkei*. All three of these *Euplotes* spp. are large (over 100 μm long) and have at least 40 membranelles in the AZM, whereas *E. parkei* is small (under 50 μm long) and has less than 20 membranelles. Furthermore, the shapes of the macronuclei differ and all three species have many more dorsal cilia than *E. parkei*.

It is evident therefore that *Euplotes parkei* differs from all previously described species of *Euplotes* and the differences are considered to be sufficiently distinct for this organism to be designated as a separate species. It is named *Euplotes parkei* after Dr Mary Parke of the Marine Biological Station Plymouth who first isolated and cultivated this hypotrich. Following the revised classification of the Committee on Taxonomic Problems of the Society of Protozoology (Honigberg *et al.*, 1964), *Euplotes parkei* is placed into class Ciliatea Perty, 1852, order Hypotrichida Stein, 1859, family Euplotidae Ehrenberg, 1838.

ACKNOWLEDGEMENTS

The author is indebted to Mrs J. E. Dorahy, Department of Zoology, British Museum (Natural History), for her technical assistance, to Miss B. J. West, NERC Culture Centre for Algae and Protozoa, who made the original silver preparations of *Euplotes parkei*, to Mr A. Cockburn, Water Pollution Research Laboratory of the Department of the Environment, who isolated *E. affinis*, to Miss M. Yeoh, Maple Lodge Sewage Treatment Works, Rickmansworth, who supplied samples of activated sludge containing *E. moebiusi* and to Mr R. Harris, Department of Zoology, British Museum (Natural History), for his aid and advice on the preparation of ciliates for scanning-electron microscopy.

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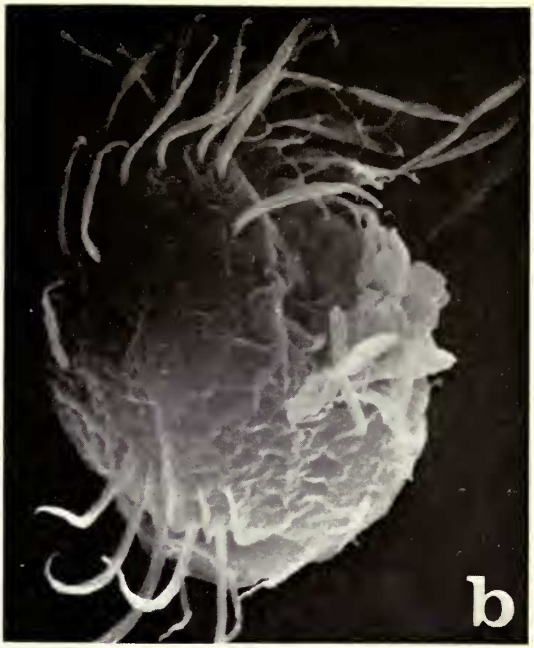
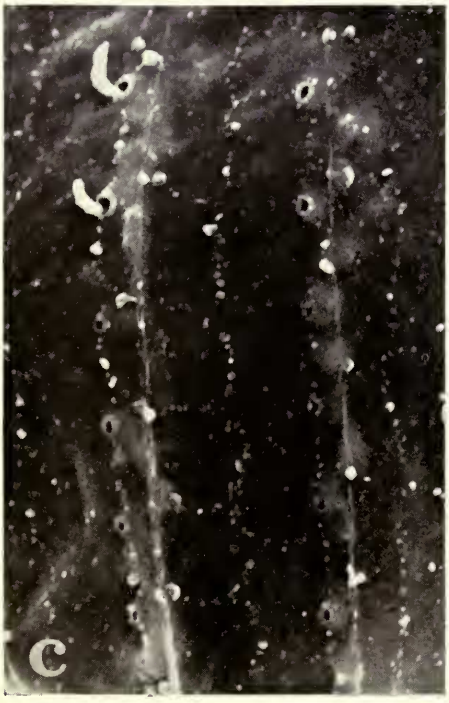
PLATE 1

Scanning-electron micrographs of *Euplotes parkei*

a. Ventral view showing ridges and cirri. Cell fixed in Parducz's (1967) fixative.
b. Ventral view of a 9 frontoventral cirri variant. Cell killed and fixed following the methods of Curds *et al.* (1974).
c. Dorsal view showing the outline of the dorsal argyrome as specks.
d. Dorsal view showing ridges, dorsal cilia and outline of argyrome.


a


b


c


d